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Characterization of acid-signaling in rat vagal pulmonary sensory neurons

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Abstract

Local tissue acidosis frequently occurs in airway inflammatory and ischemic conditions. The effect of physiological/pathophysiological-relevant low pH (7.0–5.5) on isolated rat vagal pulmonary sensory neurons was investigated using whole-cell perforated patch-clamp recordings. In voltage-clamp recordings, vagal pulmonary sensory neurons exhibited distinct pH sensitivities and different phenotypes of inward current in responding to acidic challenge. The current evoked by lowering the pH of extracellular solution to 7.0 consisted of only a transient, rapidly inactivating component with small amplitude. The amplitude of this transient current increased when the proton concentration was elevated. In addition, a slow, sustained inward current began to emerge when pH was reduced to below 6.5. The I-V curve indicated that the transient component of acid-evoked current was carried predominantly by Na⁺. This transient component was dose-dependently inhibited by amiloride, a common blocker of acid-sensing ion channels (ASICs), whereas the sustained component was significantly attenuated by capsazepine, a selective antagonist of transient receptor potential vanilloid receptor subtype-1 (TRPV1). The two components of acid-evoked current also displayed distinct recovery kinetics from desensitization. Furthermore, in current-clamp recordings, transient extracellular acidification depolarized the membrane potential and generated action potentials in these isolated neurons. In summary, our results have demonstrated that low pH can stimulate rat vagal pulmonary sensory neurons through the activation of both ASICs and TRPV1. The relative roles of these two current species depend on the range of pH and vary between neurons.

Keywords

ASICs; TRPV1; low pH; lung; vagus

INTRODUCTION

Pulmonary interstitial acidosis develops when the production of CO₂ is exceedingly high and/or the elimination of CO₂ from the lungs is hindered. Such changes can occur in both physiological (e.g., excise) and pathophysiological (e.g., chronic obstructive pulmonary disease) conditions (6,43). Excessive production of lactic acid also occurs commonly during anaerobic metabolism such as during tissue ischemia or hypoxia. A recent study from our laboratory demonstrated that pulmonary C-fibers were activated by a bolus intravenous injection of lactic acid that lowered the pH in pulmonary venous blood to ~7.1 (17). The fact that similar responses of C-fibers were also evoked when the same molar concentration of other types of acids (formic acid, glycolic acid, etc) with almost identical pK_a (~3.8) as lactic acid

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were injected suggests a major role of H^+ ions in this action. However, we could not draw a more definitive conclusion because acid solution is also known to induce local release of potent autacoids that can activate these afferents (14).

Furthermore, local tissue acidosis is known to occur in a number of pathophysiological conditions such as inflammation, ischemia and carcinogenesis, in which extracellular pH (pH_o) can decrease by more than two pH units (4,30,42). Decrease of pH_o is known to activate acid-sensing ion channels (ASICs) in both peripheral sensory and central neurons (2,21,39, 42). ASICs, a newly described class of ligand-gated channels, have been suggested to play important roles in various physiological/pathophysiological conditions, from sensory transmission (such as nociception, mechanosensation, and taste) to ischemia, retinal function, and learning-memory (7,21,25,34,45). In addition, it has been demonstrated that protons can also directly activate transient receptor potential vanilloid receptor subtype-1 (TRPV1) (9, 19). The contributions of ASICs and/or TRPV1 to acid signaling have been recently demonstrated in the nociceptors (2,5,10,13,28,33,36,44). However, little is known about the relative roles of these two types of ion channels in the response to different levels of acidity in vagal pulmonary sensory neurons.

In light of the existing knowledge described above and the information that is currently lacking, this study was carried out to characterize the acid-signaling properties in rat vagal pulmonary sensory neurons by using whole-cell perforated patch-clamp recordings.

MATERIALS AND METHODS

Labeling vagal pulmonary sensory neurons with DiI

Cell bodies of vagal sensory nerves arising from airways and lungs reside in nodose and intracranial jugular ganglia. These sensory neurons were identified by retrograde labeling from the lungs by using the fluorescent tracer DiI as described previously (15). Young adult Sprague-Dawley rats (~160 g) were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg) and intubated with a polyethylene catheter (PE 150) with its tip positioned in the trachea above the thoracic inlet. DiI was initially sonicated and dissolved in ethanol, diluted in saline (1% ethanol v/v), and then instilled into the lungs (0.2 mg/ml; 0.2 ml $\times$ 2) with the animal's head tilted up at $\sim 30^\circ$.

Isolation and culture of nodose and jugular ganglion neurons

After 7–10 days, an interval previously determined to be sufficient for DiI to diffuse to the cell body, the rats were anesthetized with halothane inhalation and decapitated. The head was immediately immersed in ice-cold Hank's balanced salt solution. Nodose and jugular ganglia were extracted under a dissecting microscope and placed in ice-cold Dulbecco's minimal essential medium/F12 (DMEM/F12) solution. Each ganglion was desheathed, cut into ~ 10 pieces, placed in 0.125% type IV collagenase, and incubated for 1 h in 5% CO_2 in air at $37^\circ C$. The ganglion suspension was centrifuged ($150 \times g$, 5 min) and supernatant-aspirated. The cell pellet was resuspended in 0.05% trypsin in Hanks' balanced salt solution for 5 min and centrifuged ($150 \times g$, 5 min); the pellet was then resuspended in a modified DMEM/F-12 solution (DMEM/F-12 supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg /ml streptomycin, and 100 μM MEM nonessential amino acids) and gently triturated with a small bore fire-polished Pasteur pipette. The dispersed cell suspension was centrifuged ($500 \times g$, 8 min) through a layer of 15% bovine serum albumin to separate the cells from the myelin debris. The pellets were resuspended in the modified DMEM/F-12 solution supplemented with 50 ng/ml 2.5S nerve growth factor, plated onto poly-L-lysine-coated glass coverslips, and then incubated overnight (5% CO_2 in air at $37^\circ C$).

Electrophysiology

Whole-cell perforated patch-clamp recordings were carried out as described previously (15). Briefly, the coverslip containing the attached cells was placed in the center of a small recording chamber (0.2 ml) that was perfused by gravity-feed (VC-6 perfusion valve controller; Warner Instruments, Hamden, CT) with standard extracellular solution (ECS). The chemical stimulants were applied by a pressure-driven drug delivery system (ALA-VM8; ALA Scientific Instruments, Westbury, NY), with its tip positioned to ensure that the cell was fully within the stream of the injectate. Recordings were made in the whole cell perforated patch configuration (50 $\mu\text{g/ml}$ gramicidin) using Axopatch 200B/pCLAMP 9.0 (Axon Instruments, Union City, CA). The data were acquired at 5 kHz and filtered at 2 kHz. The series resistance was usually in the range of 4–8 M Ω and was not compensated. The resting membrane potential was held at -70 mV except otherwise indicated. The experiments were performed at room temperature ($\sim 22^\circ\text{C}$).

Solutions and chemicals

Standard ECS contained (in mM): 136 NaCl, 5.4 KCl, 1.8 CaCl_2 , 1 MgCl_2 , 0.33 NaH_2PO_4 , 10 glucose, 10 HEPES, pH at 7.4. For solutions with $\text{pH} \leq 6.0$, MES was used instead of HEPES for more reliable pH buffering. The intracellular solution contained (in mM): 92 potassium gluconate, 40 KCl, 8 NaCl, 1 CaCl_2 , 0.5 MgCl_2 , 10 EGTA, 10 HEPES, pH at 7.2.

DiI were purchased from Molecular Probes (Eugene, OR). DMEM/F12, trypsin and nerve growth factor were obtained from Invitrogen (Carlsbad, CA). All other chemicals were obtained from Sigma Chemical (St. Louis, MO). Stock solution of capsaicin (1 mM) was prepared in a vehicle of 10% Tween80, 10% ethanol, and 80% ECS. Stock solutions of amiloride and capsaizipine were prepared in dimethyl sulfoxide at the concentration of 500 and 20 mM, respectively. The solutions of these chemicals at desired concentrations were prepared daily by dilution with ECS. No detectable effect of the vehicles of these chemical agents was found in our preliminary experiments.

Statistical analysis

Data were analyzed by a one-way analysis of variance (ANOVA). When the ANOVA showed a significant interaction, pair-wise comparisons were made with a *post hoc* analysis (Fisher's least significant difference). Results were considered significant when $P < 0.05$. Data are means $\pm$ SE.

RESULTS

A total of 93 pulmonary sensory neurons isolated from nodose and jugular ganglia of 23 rats were studied. The whole-cell capacitances of these neurons were in the range of 11.1–42.1 pF (average: 22.5 ± 0.8 pF; $n = 93$); the majority of them (83 out of 93) were small in size (capacitance ≤ 30 pF). The average resting membrane potential of these neurons was -64.4 ± 1.3 mV (range: -50 to -82 mV; $n = 33$).

Characteristics of acid-evoked inward current in rat vagal pulmonary sensory neurons

At the holding potential of -70 mV, a rapid drop of pH_o from 7.4 evoked a transient, rapidly inactivating inward current (Fig. 1) which could be further differentiated based on activation and inactivation kinetics into a fast type (e.g., Fig. 1B) and slow type (e.g., Fig. 1C). In some of the neurons, the transient inward current was followed by a sustained component that did not desensitize during the presence of the acidic stimulus (e.g., Fig. 1, A and B); whereas a small subsets of neurons showed only a sustained current upon the acid challenge without a transient component (e.g., Fig. 1D).

As shown in Table 1, rat vagal pulmonary sensory neurons exhibited distinct pH sensitivities. A mild pH_o drop to 7.0 activated 45.2% of these sensory neurons, and pH 6.5, 6.0, and 5.5 were able to activate 83.1%, 91.5%, and 92.5% of these neurons, respectively. In response to acidic challenge, pulmonary sensory neurons also showed different phenotypes of inward currents. The current evoked by pH 7.0 consisted of only the transient component with small amplitude (e.g., Fig. 1, *B and C*). The amplitude of this transient current increased when the proton concentration increased (Table 1). Interestingly, the transient current apparently reached the maximum amplitude even at pH 6.5 in some neurons (e.g., Fig. 1A). In contrast, the sustained current increased its amplitude in a larger percentage of neurons when a lower pH_o was applied (e.g., Fig. 1, *A and B*; Table 1). Our data did not indicate a close association between the cell size of these sensory neurons and their pH sensitivities (Table 1).

In 45 of these neurons, we also tested their responses to capsaicin. Rapid application of capsaicin (1 μM ; 2–6 s) evoked an inward current in 31 (68.9%) neurons; the average amplitude of which was 1183.4 ± 250.2 pA. The average whole-cell capacitance of these capsaicin-sensitive neurons was 20.9 ± 1.0 pF.

Sodium is the charge-carrying ion of the transient component of acid-evoked current in rat vagal pulmonary sensory neurons

The I-V curve was constructed by plotting the peak amplitude of the transient component of the current evoked by a pH_o drop from 7.4 to 6.5 (or 6.0, 5.5) at different membrane potentials. Neurons were voltage-clamped initially at -80 mV and then changed at a step of $+20$ mV every 5 min. At each of these holding potentials, the current activated by the same low pH challenge applied for 6 s was recorded.

As shown in Fig. 2, the transient component of acid-evoked current in these pulmonary sensory neurons had a linear I-V relationship with a reversal potential at ~ 65.4 mV ($n = 8$), which is close to the theoretical Na^+ equilibrium potential ($E_{\text{Na}} = 71.6$ mV with extra- and intra-cellular solutions containing 136 and 8 mM Na^+), indicating that the transient current evoked by low pH was selective for Na^+ ions.

Effects of amiloride and capsazepine on acid-evoked current in rat vagal pulmonary sensory neurons

To determine the contribution of ASICs to acid-evoked currents, we applied amiloride, a known blocker of ASICs. As illustrated in Fig. 3, 2-min pretreatment with amiloride dose-dependently attenuated the transient current evoked by low pH with EC_{50} at 32.6 μM . High dose of amiloride (1 mM) significantly inhibited the peak transient current to a mean of $34.1 \pm 8.9\%$ of the control responses (Fig. 3B; $n = 7$). The effect of amiloride was reversible after 10-min washout in all seven neurons tested (e.g., Fig. 3A).

We also examined the relative contribution of TRPV1, another channel gated by protons (9, 19), to the acid-evoked current in these pulmonary sensory neurons. Pretreatment with capsazepine (10 μM ; 2 min), a specific antagonist of TRPV1, significantly attenuated the sustained (e.g., Fig. 4, *A, B and E*), but not the transient (e.g., Fig. 4, *A, C and E*) component of the current evoked by low pH. Consistent with what we demonstrated previously (15), this dose of capsazepine was able to completely abolish the capsaicin-evoked inward current in these sensory neurons (Fig., 4, *D and F*). Both acid- and capsaicin-evoked currents were reversible 20–30 min after the termination of capsazepine treatment (Fig. 4).

In fourteen pulmonary sensory neurons, we examined the effects of amiloride and capsazepine in the same sensory neurons. As shown in Fig. 5, the transient component of acid-evoked current was primarily sensitive to amiloride, whereas the sustained component of which was

mainly sensitive to capsazepine. There seemed no apparent interactions between these two chemicals when applied to the neurons simultaneously (Fig. 5).

Recovery time course of acid-evoked current in rat vagal pulmonary sensory neurons

To test the recovery from acid-induced desensitization, neurons were exposed to a low pH stimulus (6.5–5.5) for 6 s, a duration sufficient to completely desensitize the transient component of acid-evoked current. Neurons were then bathed in the standard ECS (pH 7.4) for progressively shortening intervals before they were again challenged with the same low pH solution. The amplitudes of both components of the subsequent acid-evoked current were then compared with those of their corresponding controls, respectively. Our results showed that the transient current required 10–30 s for a complete recovery ($n = 7$); whereas at least 60–120 s had to elapse before the sustained current fully recovered from desensitization ($n = 4$) (Fig. 6, *A* and *B*).

To compare the recovery time course between acid- and capsaicin-evoked currents, in a group of five neurons, we challenged cells with capsaicin with the similar protocol as described for low pH. Short application (2–4 s) of 1 μ M capsaicin evoked a comparable or a slightly smaller inward current in the same neurons, whereas the full recovery time for capsaicin (6–8 min) was much longer compared to that for low pH (Fig. 6, *C* and *D*).

Effect of acid on the excitability of rat vagal pulmonary sensory neurons

This series of study was carried out to test whether depolarization caused by transient extracellular acidification was sufficient to trigger action potentials in these isolated neurons. As shown in Fig. 7A, pH 7.0 failed to evoke a detectable inward current in this nodose ganglion neuron, but it induced a clear depolarization of the membrane potential (rest membrane potential: -71 mV). When the pH_o was dropped to 6.5, action potentials were generated. In this particular neuron, when pH_o decreasing from 6.5 to 6.0 and 5.5, the amplitudes of both the transient and sustained components of acid-evoked current in voltage-clamp recordings increased correspondingly. Similarly, in current-clamp mode, the temporal pattern of membrane depolarization also exhibited the transient and sustained components, coincided with those displayed in the voltage-clamp mode. The pattern of the action potentials evoked by capsaicin (e.g., Fig. 7B) or current injection (e.g., Fig. 7C) in the same neurons differed from that by low pH ($n = 5$). Moreover, when ≥ 10 s elapsed, the depolarization/action potentials evoked by acidic challenge in current-clamp recordings was almost fully recovered from desensitization in all four neurons tested (e.g., Fig. 7D).

DISCUSSION

Our results showed that physiological/pathophysiological-relevant low pH (7.0–5.5) activated rat vagal pulmonary sensory neurons. In voltage-clamp recordings, these sensory neurons exhibited distinct pH sensitivities and different phenotypes of inward currents in response to low pH challenge. About half of these neurons responded to pH 7.0 with a small, transient current. The amplitude of this transient current increased when the proton concentration was elevated. This transient current was predominantly carried by Na^+ , and was dose-dependently blocked by amiloride, indicating that it was mediated by ASICs. In addition, a slow, sustained inward current following the transient component began to emerge when $pH \leq 6.5$ was applied. This sustained current was significantly attenuated by capsazepine, indicating that it was mediated primarily through TRPV1. In current-clamp recordings, transient extracellular acidification depolarized the membrane potential and generated action potentials in these sensory neurons. Furthermore, the inward currents and depolarization/action potentials mediated by these two different acid-activating channels displayed distinct activation, inactivation and recovery properties.

ASICs, a novel class of ligand-gated cation channels activated by protons, belong to the amiloride-sensitive degenerin/epithelial sodium channel superfamily (3,12,21,39,41). So far, four genes encoding six ASIC subunits have been cloned. Four of these subunits can form functional homomultimeric channels (45). The pH of half-maximal activation ($\text{pH}_{0.5}$) of these channels differs: ASIC1a, $\text{pH}_{0.5} = 6.2$ (39); ASIC1b, a splice variant of ASIC1a, $\text{pH}_{0.5} = 5.9$ (10); ASIC2a, $\text{pH}_{0.5} = 4.4$ (29,40); and ASIC3, $\text{pH}_{0.5} = 6.5$ (34,38). Neither ASIC2b nor ASIC4 can form functional homomeric channel (1,26). Whereas ASIC1a, ASIC1b, and ASIC2a display transient activation characteristics, ASIC3 responds to acid stimuli biphasically, with a quick desensitizing and a late sustained currents (16). ASICs have been shown to be expressed throughout the neurons of mammalian central and peripheral nervous systems (2,21,42). Like other ligand-gated ion channels, functional ASICs can be formed by homomultimers as well as heteromultimers (5,39). Different homomeric and heteromeric ASICs are known to have distinct pH sensitivity, ion selectivity, and channel kinetics (16). In dorsal root ganglion neurons, where many subtypes are expressed, native ASICs are believed to be heteromultimeric (2,5). Our results have demonstrated for the first time that the acid-evoked inward currents in these pulmonary sensory neurons exhibited the properties of ASIC1 (e.g., Fig. 1B), ASIC2 (e.g., Fig. 1C), and ASIC3 (e.g., Fig. 1, A and B)-like responses. However, we do not have more definitive evidence of their expressions in these neurons. The relative narrow physiological/pathophysiological range of low pH (7.0–5.5) applied in this study further limited our ability to measure the $\text{pH}_{0.5}$ in these native neurons. Nevertheless, the reversal potential of the transient component, estimated from the current-voltage relationship (Fig. 2), was close to the theoretical Na^+ equilibrium potential. This Na^+ selectivity, together with amiloride sensitivity (Figs. 3 and 5) and rapid recovery from desensitization (Fig. 6) have provided the critical evidence of the activation of ASICs in these vagal pulmonary sensory neurons.

In this study, a slow, sustained inward current began to emerge when the extracellular pH is lowered to ≤ 6.5 , and the response did not inactivate during the presence of the acidic stimulus (e.g., Fig. 1, A, B and D). The percentage of the neurons exhibiting this current and the amplitude of the current increased when the extracellular pH is reduced further; for example, when pH 5.5 (4–6 s) was applied, about 62% of neurons showed the sustained current (Table 1). Coincidentally, a similar percentage of neurons ($\sim 69\%$) responded to capsaicin (1 μM ; 2–6 s) challenge. In contrast to its effect on the acid-evoked transient current, amiloride did not significantly affect the sustained current in these sensory neurons (Fig. 5). Instead, the latter was markedly attenuated by capsazepine (Figs. 4 and 5), a selective TRPV1 antagonist, indicating that it was mainly mediated by the activation of TRPV1 in these sensory neurons. Indeed, it has been known that, in addition to ASICs, protons can also directly activate TRPV1 (9,19). The contribution of TRPV1 to the acid signaling has been extensively studied recently in the cutaneous nociceptive neurons (9,35,36,37), where it has been shown that TRPV1 contributes to a major part of acid-induced nociception, especially the generation of the persistent pain under more severe acidification (8,36).

Our results have shown that the ASICs-mediated transient component of acid-evoked inward current recovered completely from desensitization within 30 s, whereas more than 60 s was required for the TRPV1-mediated sustained component to reach a full recovery (Fig. 6, A and B). It is noteworthy that the latter was still significantly shorter than that for the capsaicin-evoked current (at least 6 min; Fig. 6, C and D) which was known to be solely mediated by TRPV1 (e.g., Fig. 4, D and F). This difference in the recovery time course between acid- and capsaicin-evoked TRPV1 responses may be, in part, due to the difference in the amplitudes of the peak currents evoked by these two chemical stimuli (Fig. 6). An alternative explanation could be that this difference was due to the different binding sites and different activation and inactivation kinetics of these two chemicals on this ion channel. It has been known that capsaicin binds to an intracellular site to activate TRPV1 (8), whereas proton has been

suggested to interact with an extracellular site(s) on the channel complex (35). Indeed, several candidate sites located within putative extracellular loops of TRPV1 has been identified for such interactions, with two glutamate residuals E648 and E600 of particular interest (8,18). Our study has also shown that low pH can cause the depolarization of membrane potential and generation of action potentials in these pulmonary sensory neurons. Interestingly, the acid-evoked action potentials always fired at the transient (peak) but not the sustained (plateau) phase of the depolarization (e.g., Fig. 7, A and D). A similar observation has also been reported in acid-induced activation of dorsal root ganglion neurons (27). It is believed that the TTX-resistant Na⁺ channels that are expressed in distinctly higher percentage in capsaicin-sensitive pulmonary neurons (22), plays a critical role in determining the activating properties of these neurons (23,27).

The present study has shown that pH 5.5 stimulated the majority of pulmonary sensory neurons (92.5%) through the activation of ASICs and/or TRPV1, which is in agreement with the observation made by Kollarik and Undem (20). These investigators recorded the extracellular action potential in an isolated airway nerve preparation, and demonstrated that the activation of guinea pig airway afferents by low pH was mediated by both slowly (TRPV1 dependent) and rapidly (TRPV1 independent) inactivating mechanisms. However, our results did not indicate a significant difference between pulmonary sensory neurons isolated from nodose and jugular ganglia in responding to acid challenge (not shown); nor did our data show a significant difference in cell size between the groups of neurons with different pH sensitivities (Table 1). Whether the differences in animal species (rat vs guinea pig) and experimental approaches contribute to the discrepancy between these two studies is not known.

It should be noticed that our data of acid-sensing properties in the present study was obtained from isolated pulmonary sensory neurons; whether there is a difference in the sensitivity to low pH between the sensory terminal and its neuronal soma remains to be determined. However, it has been recognized that airway acidification induces cough and bronchoconstriction in humans and laboratory animals which has been suggested to result mainly from the release of neuropeptides from the acid-evoked activation of bronchopulmonary C-fibers (20,31). Indeed, the majority of the vagal pulmonary afferent nerves are non-myelinated (C-) fibers. Stimulation of these C-fiber afferents is known to elicit a number of reflex responses mediated through the central and/or autonomic nervous systems, including bronchoconstriction, mucus hypersecretion, cough, dyspneic sensation and bronchial vasodilatation (11,24). In addition, sensory neuropeptides such as tachykinins (e.g., substance P, neurokinin A) and calcitonin gene-related peptide are synthesized in the cell bodies of these C-fibers and released locally from the sensory terminals upon stimulation. These peptides are known to act on a number of effector cells (e.g., airway smooth muscles, cholinergic ganglia, inflammatory cells, mucous glands) and produce potent local effects such as bronchoconstriction, extravasation of macromolecules, and edema of airway mucosa in various species including humans (24,32).

In summary, our results demonstrate that low pH can activate the majority of rat vagal pulmonary sensory neurons. The pH sensitivity and pattern of responses vary between neurons; and the acid signaling in these neurons is mediated through the activation of ASICs and TRPV1. Since the pH levels used in this study are well within the range of those reported for various pathophysiological conditions such as tissue inflammation and ischemia (4,30), these findings may provide the information for elucidating the acid-signaling mechanisms in various airway diseases in which extracellular acidification is known to occur.

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Fig. 1.

Representative acid-evoked whole cell inward currents in rat vagal pulmonary sensory neurons. Low pH with increasing proton concentrations were applied for 6 s (as indicated by the horizontal bars) to four different jugular ganglion neurons. The cell capacitances for the neurons in A, B, C, and D were 24.2, 23.1, 15.6, and 11.1 pF, respectively. Note distinct pH sensitivities and different phenotypes of inward currents in response to acidic challenges.

Fig. 2.

Acid-evoked transient inward currents at different membrane potentials in rat vagal pulmonary sensory neurons. *A*: representative records illustrating pH 6.5-evoked transient currents in a jugular neuron (23.2 pF) voltage clamped at the membrane potentials indicated in the figure. These membrane voltages were held for 90 s before the acid application to ensure any voltage-gated conductance were inactivated. V_m , membrane potential. *B*: current-voltage relationship of low pH (6.5–5.5; 6 s)-evoked transient currents from eight sensory neurons; the reversal potential was estimated at ~65.4 mV. I , peak inward current evoked by acid application. Data represent means $\pm$ SE.

Fig. 3.

Effect of amiloride on the acid-evoked transient inward currents in rat vagal pulmonary sensory neurons. *A*: representative records illustrating pH 6.5-evoked transient currents in the absence and presence of a 2-min pretreatment of increasing concentration of amiloride in a jugular neuron (36.5 pF). *B*: group data showing the inhibitory effect of amiloride on low pH (6.5–5.5; 2–6 s) -evoked transient currents from seven sensory neurons; Chapman fit: EC_{50} , 32.6 μM .

Fig. 4.

Effect of capsazepine on the acid- and capsaicin-evoked currents in rat vagal pulmonary sensory neurons. *A, B* and *C*: representative records illustrating the effect of capsazepine (CZP; 10 μ M; 2 min) pretreatment on pH 5.5 (4–6 s)-evoked inward currents in nodose (29 pF), jugular (15.6 pF), and nodose (18.6 pF) neurons, respectively. *D*: representative records illustrating the effect of CZP (10 μ M; 2 min) on capsaicin (Cap; 1 μ M, 2 s)-evoked currents in a jugular neuron (15.9 pF). *E* and *F*: group data showing the effect of CZP on the inward currents evoked by low pH (6.5–5.5; 4–6 s) and capsaicin (1 μ M; 2–4 s), respectively. * $P < 0.05$ as compared with the corresponding control. Data represent means $\pm$ SE.

Fig. 5.

Effects of amiloride and capsazepine on the acid-evoked currents in the same rat vagal pulmonary sensory neurons. *A*: representative records illustrating the effects of amiloride (100 μ M; 2 min) and capsazepine (CZP; 10 μ M, 2 min) on pH 5.5 (6 s)-evoked inward currents in a jugular neuron (23.1 pF). *B*: group data showing the effects of 2-min pretreatment with amiloride (100 μ M) and CZP (10 μ M) on both transient and sustained components evoked by low pH (6.5–5.5; 6 s). * $P < 0.05$ as compared with the corresponding control. Data represent means $\pm$ SE.

Fig. 6.

Different recovery kinetics of acid- and capsaicin-evoked currents in rat vagal pulmonary sensory neurons. *A* and *C*: inward currents evoked by repeated application of low pH (5.5; 6 s) and capsaicin (Cap; 1 μ M, 3 s), respectively, at different intervals in the same pulmonary sensory neuron (nodose; 24.7 pF). *B* and *D*: group data showing the different recovery kinetics of acid- (6.5–5.5; 6 s) and capsaicin- (1 μ M; 2–4 s) evoked currents in these sensory neurons, respectively. * $P < 0.05$ as compared with the corresponding control (Con). Data are means $\pm$ SE.

Fig. 7.

Changes in membrane potential (current-clamp) and inward current (voltage-clamp) in response to different stimuli in rat vagal pulmonary sensory neurons. *A* and *B*: acid- (pH 7.0–5.5; 6 s) and capsaicin- (Cap; 1 μ M, 6 s), respectively, evoked changes in membrane potential (V_m ; upper traces; current clamp) and inward current (lower traces; voltage clamp). *C*: action potentials (upper trace) evoked by a 50 pA current injection (198-ms duration; lower trace). *A*, *B* and *C* were recorded from a single nodose neuron (23.3 pF). *D*: action potentials (upper traces) and inward currents (lower traces) evoked by pH 5.5 (6 s) challenge at different intervals in a jugular neuron (30.1 pF). Insets, the action potentials triggered by various stimuli are shown on a large scale.

Table 1
Characteristics of acid-evoked inward currents in rat vagal pulmonary sensory neurons

	Responding neurons (%)	Neurons with transient current (%)	Peak amplitude of transient current (pA)	Neurons with sustained current (%)	Peak amplitude of sustained current (pA)	Neurons with both components (%)	Cap. of responding neurons (pF)
pH 7.0	45.2 (28/62)	100 (28/28)	41.9 ± 11.1	0 (0/28)	0 ± 0	0 (0/28)	24.4 ± 1.0
pH 6.5	83.1 (59/71)	89.8 (53/59)	398.2 ± 82.1	25.4 (15/59)	106.8 ± 88.6	13.6 (8/59)	22.6 ± 0.8
pH 6.0	91.5 (65/71)	81.5 (53/65)	634.9 ± 160.4	46.2 (30/65)	127.5 ± 87.3	30.8 (20/65)	21.9 ± 0.8
pH 5.5	92.5 (74/80)	82.4 (61/74)	671.7 ± 179.4	62.2 (46/74)	418.3 ± 112.9	44.6 (33/74)	22.2 ± 0.7

Low pH solutions were applied to the neurons shortly (4–6 s) in whole cell perforated voltage clamp configuration. The membrane potential was held at −70 mV. At least 5 min elapsed between two consecutive low pH challenges. Cap., capacitance. Data are means ± SE.